

STUDY ON SEED MYCOFLORA OF DIFFERENT VARIETIES OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.)

YARIELYN SAIO & MAMTAJ SAHANA DKHAR

Microbial Ecology Laboratory, Centre of Advanced Studies in Botany, Department of
Botany North Eastern Hill University, Shillong, Meghalaya

ABSTRACT

Seeds of eight French bean varieties were selected to study the occurrence of seed mycoflora. Isolation was done by Direct Plate method (Warcup, 1950) and Dilution Plate method (Johnson and Curl, 1972) using Rose Bengal Agar medium. A total of 17 genera, 31 fungal species and 2 sterile mycelia were isolated from the seed varieties. The most abundant species were found to be species of *Aspergillus*, *Cladosporium* and *Penicillium* while the least abundant were species of *Actinomucor*, *Eupenicillium*, *Fusarium*, *Nectria* and *Rhizopus*. The highest colony form unit (CFU) was observed in FB 62 and FB 61 in Direct Plate method and Dilution Plate method respectively, while the lowest CFU was observed in Manipur variety in Direct Plate method and Naga Local variety in Dilution Plate method. From the present study, it was observed that a large number of fungal species colonized all the seed varieties studied which may cause deterioration in the seed quality and poor performance of the crop in the field. It was also observed that the Direct Plate method was more suitable for the isolation of seed microflora in comparison to Dilution Plate method.

KEYWORDS: Seed Mycoflora, French Bean, Direct Plate Method & Dilution Plate Method

Received: Jun 24, 2017; **Accepted:** Jul 12, 2017; **Published:** Jul 21, 2017; **Paper Id:** IJASRAUG201753

INTRODUCTION

French bean or common bean (*Phaseolus vulgaris* L.) is considered as one of the most important legume crops grown worldwide, and is consumed both as a pulse and green vegetable. It contains many essential nutrients, but it is well known for its protein rich (23%) seeds. French beans are usually being grown as winter crop, however, in hilly regions; the crop is cultivated throughout the year except winter. In Meghalaya and other places situated in the mid-hills of north-eastern region, French beans are grown from the month of March to December when highest summer temperature reaches up to 32°C (Shivastava *et al.*, 2012).

In agricultural production, the most important input is the seed quality which greatly influences the survival and yield of the crop in the field (Eliud *et al.*, 2010). The viable seed is a source of a new plant and contains genetic material in compact form that is well protected from extraneous factors. The quality of seeds depends on many factors such as viability of seeds, field deterioration and other environmental factors. Besides, microorganisms also play a dominant role in decreasing quality and longevity of the seeds. After harvesting, seeds are stored at different storage conditions. The unfavorable storage conditions can lead to the colonization of various microorganisms like bacteria, viruses, nematodes and fungi in these seeds. Among these microorganisms, fungi are the largest group (Javaid *et al.*, 2006).

The influence of microorganisms on the overall health, germination and final crop stand in the field have brought about the importance in the study of seed mycoflora. The dissemination of plant pathogens, disease

establishment and deterioration of seed in storage has been greatly attributed to the utilization of infected seed during sowing period (Agarwal, 1974, Shah and Jain, 1993). During maturation, harvesting and storage, the French bean seeds are colonized by different microorganisms (Neergaard, 1977) and are carried either on the surface or within itself. Some of the seed-borne fungi were found to be the causal organisms of seed rot, decrease in seed germination and destruction to the plant in the field (Bolkan *et al.*, 1975 and Elarosi 1993). Seeds are known to be the main carriers of some important seed-borne diseases caused by microorganisms, which later on cause diseases to the plant and results in considerable losses in yields. In view of this, our present study deals with the mycoflora associated with the seeds of different varieties of French bean growing in Meghalaya.

MATERIALS AND METHODS

For the present investigation, 8 varieties of French bean seeds namely, Phyrngop, Manipur, Naga Local, FB 19, FB 61, FB 62, Director-1 and Director-3 were collected from Ri Bhoi District (ICAR, Umiam), Meghalaya. The collected seeds were stored in storage bags under laboratory conditions. Isolation of the seed mycoflora was done following two isolation methods viz. Direct Plate method (Warcup, 1950) and Dilution Plate method (Johnson and Curl, 1972) using Rose Bengal Agar (RBA) medium. For each variety, isolation was carried out separately for determining the range of mycoflora. The Colony form units (CFU) of fungi per gram sample were calculated on the dry weight basis. The fungal species were identified on the basis of their morphology and reproductive structures, consulting monographs of Gilman (1956), Burnet and Hunter (1972), Domsch *et al.* (1980); Subramaniam (1983) and Ellis (1993). The following formula was used for the determination of relative abundance of fungal species:

$$\text{Relative Abundance (\%)} = \frac{\text{Total number of colonies of individual species}}{\text{Total number of colonies of all the species}} \times 100$$

Species diversity and Species dominance were calculated using the Shannon diversity index (H) (Shannon and Weaver, 1949) and Simpson (1949) dominance index (C). One-way ANOVA analysis ($p \leq 0.05$) was also done to determine the variation in the CFU of the varieties between the two methods.

RESULTS

A total of 17 genera, 31 fungal species and 2 sterile mycelia were isolated from the eight selected seed varieties. Out of which, 12 genera belonged to Ascomycota, 1 genus to the class Basidiomycota and 4 genera belonged to Zygomycota. Thirteen genera and 25 species were isolated by Direct Plate method and 9 genera and 16 species were isolated by Dilution Plate method (Table 1). In Direct Plate method, the highest number of fungal species was isolated from Manipur variety, (12 species and 1 sterile mycelium) and the lowest number of species was isolated from FB 61 and FB 62 varieties (8 species and 1 sterile mycelium). In Dilution Plate method, the highest number of fungal species was isolated from Naga Local variety (8 species) and the lowest number of species was isolated from Manipur variety (1 species and 1 sterile mycelium).

Table 1: Fungal Species with Their Percentage Relative Abundance Isolated from the Different Varieties of French bean (*Phaseolus Vulgaris L.*) Seeds

Sl. No	Phylum	Fungal Species		Phyrgop		Manipur		Naga Local		FB 19		FB 61		FB 62		Director-1		Director-3	
		Genus name	Species Name	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
1	Ascomycota	<i>Acremonium</i>	<i>butyri</i>	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-
2			<i>cerealis</i>	-	-	-	-	-	-	-	-	-	-	-	3.2	3.3	-	1.2	-
3			<i>strictum</i>	-	-	4	-	-	-	-	-	-	-	1.6	-	-	-	-	-
4		<i>Aspergillus</i>	<i>erythrocephalus</i>	6.8	-	-	-	-	-	3	-	1.2	-	-	-	-	-	-	-
5			<i>flavus</i>	14	15	-	7.1	6.5	50	9.1	-	9.9	36	7.8	-	12	48	7.3	60
6			<i>japonicus</i>	2.8	-	-	-	1.3	-	-	-	-	-	-	-	-	4.8	-	-
7			<i>niger</i>	16	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-
8			<i>wentii</i>	-	-	8	-	-	-	3	-	2.5	-	-	-	4.9	-	3.7	-
9		<i>Cladosporium</i>	<i>cladosporioides</i>	36	25	8	7.1	56	-	-	-	53	28	13	55	34	-	-	-
10			<i>herbarum</i>	-	15	20	2.4	9.1	-	49	-	14	1.6	34	13	36	29	55	-
11		<i>Cordana</i>	<i>musae</i>	-	-	8	-	1.3	-	-	-	-	-	-	-	-	-	-	-
12		<i>Eupenicillium</i>	<i>javanicum</i>	-	-	-	-	-	-	-	-	-	3.1	-	-	-	2.4	-	-
13		<i>Fusarium</i>	<i>semitectum</i>	-	-	-	-	-	-	3	-	1.2	-	1.6	-	-	-	-	-
14		<i>Nectria</i>	<i>ventricosa</i>	-	-	-	-	1.3	-	-	-	2.5	-	-	-	-	-	-	-
15		<i>Paecilomyces</i>	<i>variotii</i>	-	-	-	2.4	-	-	-	17	-	-	-	-	-	-	-	-
16		<i>Penicillium</i>	<i>chrysogenum</i>	5	-	-	-	7.8	-	9.1	-	-	-	-	-	6.6	-	12	-
17			<i>expansum</i>	16	15	-	-	3.9	-	6.1	61	7.4	22	-	-	3.3	2.4	11	-
18			<i>janthinellum</i>	-	2.1	4	64	1.3	-	-	-	-	9.4	-	-	-	4.8	1.2	-
19			<i>jenseni</i>	2.8	29	-	20	-	-	-	-	-	-	34	-	-	9.5	-	-
20			<i>rubrum</i>	-	-	-	-	-	-	-	-	-	-	-	23	-	-	-	-
21			<i>simplicissimum</i>	-	-	-	7.1	-	-	-	-	-	-	-	-	-	-	-	-
22		<i>Phoma</i>	<i>eupyrena</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40
23		<i>Staphylotrichum</i>	<i>coccosporum</i>	-	-	4	-	1.3	-	12	5.6	1.2	-	1.6	-	6.6	-	4.9	-
24		<i>Trichoderma</i>	<i>harzianum</i>	-	-	-	-	1.3	-	-	-	-	-	-	-	-	-	-	-
25			<i>viride</i>	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-
26	Basidiomycota	<i>Minimedusa</i>	<i>polyspora</i>	-	-	-	9.5	-	-	-	-	-	-	-	-	-	-	-	-
27	Zygomycota	<i>Actinomucor</i>	<i>elegans</i>	-	-	-	-	-	-	3	-	-	-	-	-	-	-	2.4	-
28		<i>Rhizomucor</i>	<i>pustillus</i>	-	-	12	-	-	-	-	-	2.5	-	1.6	-	-	-	-	-
29		<i>Rhizopus</i>	<i>oryzae</i>	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30			<i>R. stolonifer</i>	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-
31		<i>Syncephalastrum</i>	<i>racemosum</i>	-	-	-	-	3.9	-	3	-	-	-	-	-	-	-	-	-
32	Mycelia sterilia	White sterile mycelium		-	-	-	-	5.2	-	-	-	3.6	-	-	-	3.3	-	1.2	-
33		Yellow sterile mycelium		-	-	-	-	-	50	-	-	-	-	-	6.5	-	-	-	-

Note: ‘-’ = absent, M1=Direct Plate method, M2= Dilution Plate method

Species of *Aspergillus*, *Cladosporium* and *Penicillium* were the most common fungi occurring in all the varieties of French bean seeds. The fungus *Staphylotrichum coccosporum* was isolated from 7 varieties, except Phyrgop variety, however, most of the fungal species were found to be restricted to only few of the varieties. Species of *Acremonium* were isolated from 5 varieties while *Fusarium semitectum*, *Rhizomucor pusillus* and *Syncephalastrum racemosum* could be isolated from 3 varieties. *Minimedusa polyspora* and *Phoma eupyrena* were isolated only from 1 variety ie. Naga Local and Director-3, respectively. The most abundant species isolated was found to be *Aspergillus flavus* and the least abundant was *Trichoderma harzianum*. In addition, it was also observed that some of the fungal species could be isolated by both of the isolation methods while few were isolated only by one of the methods.

The colony form units (CFU) was found to be the highest in FB 62 variety in Direct Plate method and lowest in Manipur variety, while in Dilution Plate method, highest CFU was seen in FB 61 and lowest in Naga Local (Figure 1). The species diversity in Direct Plate method was observed to be highest in Manipur variety and lowest species diversity was recorded in the Director-3 variety (Figure 2). In Dilution Plate method, highest species diversity was observed in Phyrgop variety and lowest in Naga Local variety. The species dominance was highest in Naga Local variety in both the methods and lowest species dominance was found to be in Manipur variety in Direct Plate method and Phyrgop variety in Dilution Plate method (Figure 3).

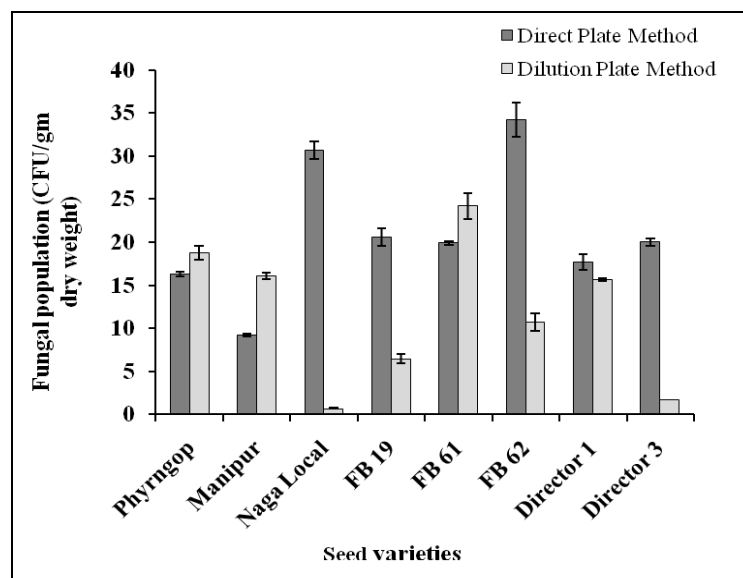


Figure 1: Fungal Population (CFU/g dry wt) of French Bean Seeds

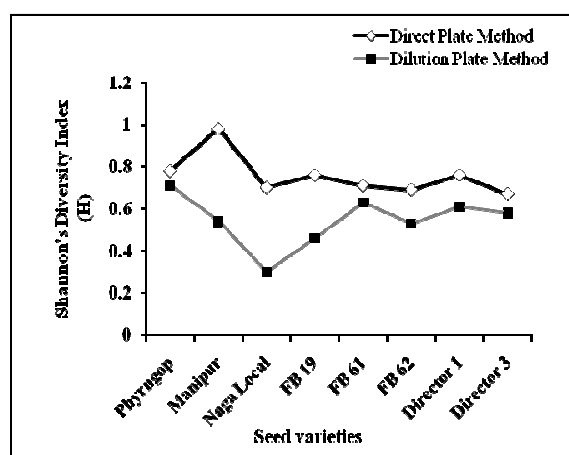


Figure 2: Shannon Diversity Index (H) of French Bean Seeds

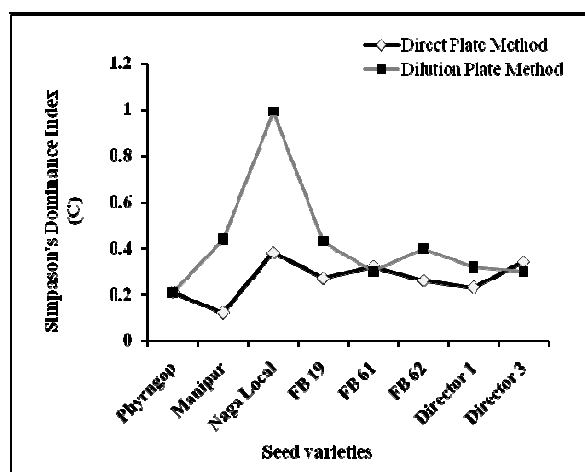


Figure 3: Simpson Dominance Index (C) of French Bean Seeds

The CFU, species diversity and species dominance was observed to be higher in the Direct Plate method than in the Dilution Plate method. The one-way ANOVA ($p \leq 0.05$), Table 2 showed a significant difference between the two methods in the CFU of FB 19 variety. However, there was no significant difference in the other varieties.

Table 2: One-Way ANOVA Analysis ($p \leq 0.05$) of the CFU of Different Seed Varieties Between the Two Methods

Source of Variation		Seed Varieties							
		Phyrngop	Manipur	Naga Local	FB 19	FB 61	FB 62	Director-1	Director-3
M1 + M2	F ratio	0.0423	0.596	2.56	10.72	0.0839	1.532	0.0942	3.981
	p level	0.847	0.483	0.185	0.031	0.787	0.2835	0.774	0.117

Note: M1=Direct Plate method, M2= Dilution Plate method

DISCUSSIONS

The difference in the number of fungal species isolated from the seed varieties of French bean may be attributed to the environmental conditions during storage and moisture content of the seeds. Another factor contributing to the differences in the isolation frequency may be difference in the nutrient requirement of the fungal species present in the seed varieties. Similar results concerning the differences in frequency of fungal isolation have also been reported by other researchers (Abdel-Hafez, 2004; Al-Shebel and El-Hussieni, 2007). Pande *et al.* (2005) stated that some fungi can infect seeds before harvest, but the survival of many fungi depends on favourable environmental factors (i.e., temperature, moisture content) and storage conditions.

It was found that species of *Aspergillus*, *Cladosporium* and *Penicillium* were recorded in all the seed varieties. This may be attributed to their dominance nature and high resistance of these species to environmental factors (i.e., storage, transportation, temperature, moisture content). Dominance of these fungal species may also be due to the fast growing nature of these fungi and availability of favourable moisture during the storage period of the seeds. Pereyra *et al.* (2008) reported that seeds stored under high moisture conditions results in dominance of fungal species of the genera *Aspergillus* and *Penicillium*. Fungi of rare occurrence reflect their more demanding nature for nutrients under unfavourable environmental conditions as suggested by Bilgrami *et al.* (1979). It may also be due to lack of competitive ability of these fungal species (Campbell, 1962).

High diversity of species in some varieties of seeds may be contributed by the presence of fungal species having wide range of nutrient requirements and capacity to tolerate varying environmental factors. Low species diversity in some varieties may be due to the presence of fungal species which have greater sensitivity to storage conditions and other environmental factors.

The colony form unit (CFU), species diversity and species dominance of most of the varieties was observed to be comparatively higher in Direct Plate method than in Dilution plate method. This may be due to the reason that in Dilution Plate method, some spores did not get dispersed into the solution, but remained adhered to the seed coat or failure of the spore masses to break. This finding is in conformity with the findings of Paul (1991).

CONCLUSIONS

From this study, it can be concluded that species of *Aspergillus*, *Cladosporium* and *Penicillium* were more dominant than the other species, which indicates the dominance of these species in the mycoflora of the French bean seed varieties studied. High isolation frequency and fungal population in the seed varieties suggested that a large number of

fungi were present in the seeds, which could later cause deterioration of the seeds, hence, resulting in poor performance of the plant. High CFU, species diversity and species dominance in Direct Plate method than in Dilution Plate method suggested that Direct Plate method can be recommended for the isolation of seed mycoflora of French bean, as the method is simple, reproducible and effective.

ACKNOWLEDGEMENTS

The authors thank the Department of Botany, North Eastern Hill University, Shillong, for providing all the facilities necessary for the study. We also thank the Division of Agronomy, ICAR, Umiam, Meghalaya, for providing the seed samples required in this study. Fellowship support by UGC is duly acknowledged.

REFERENCES

1. Abdel-Hafez, S.I.I. (2004). Mycoflora of bean, broad bean, lentil, lupine and pea seeds in Saudi Arabia. *Mycopathologia*, 88, 45-49.
2. Al-Shebel, S. & El-Hussieni, S. (2007). Susceptibility of some legume crops to *Rhizoctonia solani* AG-4 recovered from bean plants. *The international conference on the Arabian oryx in the Arabian Peninsula, the 23rd Meeting of the Saudi Biological Society*.
3. Agarwal, V.K. & Singh, O.V. (1974). Relative percentage incidence of seed borne fungi associated with different varieties. *Seed Research*, 2, 23-25.
4. Barnett, H.L. & Hunter, B.B. (1972). *Illustrated genera of Imperfect Fungi* (3rd Eds.). Burgess Publishing Company, Minneapolis, Minnesota, USA.
5. Bilgrami, K.S., Prasad, T. & Sinha, R.K. (1979). *Changes in nutritional components of stored seeds due to fungal association. Today and Tomorrow's Printers and Publishers, New Delhi*.
6. Bolkan, H.A., De Silva, A.R. & Cupertino, F.P. (1975). Fungi associated with soybean and been seeds and their control in central Brazil. *Plant Disease Reporter*, 60, 545-548.
7. Cambell, W.P. (1962). Storage of treated moistened grains, *Canada Plant Diseases Survey*, 42, 195-201.
8. Domsch, K.H., Gams, W. & Anderson, T.H. (1980). *Compendium of soil fungi*. Academic Press, London.
9. Elarosi, H. (1993). *Diseases of vegetables*. New Publishing House, Alexandria. Pp. 310.
10. Eliuda, R., Reuben, M. & Linneta, G. (2010). Longevity of Bean (*Phaseolus vulgaris*) seeds stored at locations varying in temperature and relative humidity. *Journal of Agriculture, Pure and Applied Science and Technology*, 5, 60-70.
11. Ellis, M.B. (1993). *Dematiaceous Hyphomycetes*. CAB International, Wallingford, U.K.
12. Gillman, J.C. (1957). *Manual of soil fungi* (2nd eds.). Oxford and I.B.H Publishing company (Indian reprint).
13. Javaid, A. & Anjum, T. (2006). Fungi associated with seeds of some economically important crops in Pakistan – A review. *Pacific Journal of Science and Technology*, 1, 8-9.
14. Johnson, L.F. & Curl, A.E. (1972). *Method for the research on necology of soil borne plant pathogens*. Burgess Publishing Company, Minneapolis. Pp. 249.
15. Neergaard, P. (1977). *Seed Pathology*. Vol. I & II. The Macmillan Press Ltd., London and Asingstoke. p. 1187.
16. Pande, S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W. & Crouch, J.H. (2005).

- Ascochyta* blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research*, 56, 317-332.
17. Paul, M.C. (1991). Studies on seed fungi of maize-biochemical changes in relation to fungal infection. Ph.D. Thesis, NEHU, Shillong.
 18. Pereyra, G.M.L., Pereyra, C.M., Ramírez, M.L., Rosa, C.A.R., Dalcero, A.M. & Cavaglieri, L.R. (2008). Determination of mycobiota and mycotoxins in pig feed in central Argentina. *Letters in Applied Microbiology*, 46, 555-561.
 19. Shah, R. & Jain, J. P. (1993). Seed mycoflora of mustard and its control, *Indian Journal of Mycology and Plant Pathology*, 23, 291-295.
 20. Shivastava, J.N., Dwivedi, B.P., Shukla D.N. & Dutta, U. (2012). Seed mycoflora of rajmash [*Phaseolus vulgaris* (L.)] in relation to different coloured seed coat. *International Journal of Plant Sciences*, 7 (1), 263-265.
 21. Subramaniam, C.V. (1983). *Hyphomycetes – Taxonomy and Biology*. Academic Press, London.
 22. Warcup, J.H. (1950). The soil plate method for isolating of fungi from the soils. *Nature*, 166, 117-118.

